IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.	: 10/086,477	Confirmation No.	: 3225
Applicant	: Semple *et al.*		
Filed	: 3/1/02	Attorney Docket No.:	33846/US/3
Art Unit	: 1632	Customer No.	: 32,940
Examiner	: Nguyen, D.		
Title	: COMPOSITIONS FOR STIMULATING CYTOKINE SECRETION AND INDUCING AN IMMUNE RESPONSE		

DECLARATION
Pursuant to § 1.132

I, Michael J. Hope, declare the following:

1. I have a Ph.D. in Biochemistry from the University of London, UK. I have done research in the field of lipids and liposomal delivery vehicles for thirty (30) years and published many papers in the field. My curriculum vitae is attached. I am familiar with the significant variations in the liposomal physical structure that can result from changes in composition and method of preparation. I have been employed by Inex Pharmaceuticals Corporation, the assignee of the above-referenced patent application, as a research scientist since 1994. I am personally familiar with the inventor of the Wheeler patent mentioned below, who was a colleague and co-researcher with me at Inex. I am also knowledgeable about the lipid compositions described by the Wheeler patent and the use thereof in forming lipid nucleic acid complexes as a delivery vehicle.

2. I am a co-inventor of the instant invention, and thus I have read the specification, made the lipid-encapsulated nucleic acid particles described therein, and am knowledgeable about their structure. I have also read the Office Action dated December 22, 2003 and I have read and understand each of U.S. Patent Nos.: 5,703,055 to Felgner, 6,207,646 to Krieg; 5,976,567 to Wheeler; and 6,143,716 to Meers, as well as the 1999 review article by McCluskie and the 1998 *J. Immunotherapy* article by Bei. Through my research experience and familiarity with the structure of liposomal compositions I am able to recognize the types of lipid/nucleic acid structures that would be formed by the methods

described in each of the aforementioned references in comparison to the improved lipid/nucleic acid structures taught in the instant application.

3. I understand from reviewing the Office Action that the Examiner considers the lipid/nucleic acid compositions prepared by the methods described in the cited art, particularly Felgner, Wheeler or Meers, to be the same as or equivalent to the presently-claimed compositions having nucleic acid polymers fully encapsulated in a cationic lipid particle. I believe that the Examiner's opinion is incorrect, for the reasons that follow.

4. First, the Meers reference is directed principally to peptide-lipid conjugates and their use in liposomes. Meers would appear to be of very little relevance to our application since it does not describe with any specificity the making of a lipid/nucleic acid composition, let alone the lipid-encapsulated nucleic acid compositions of our invention.

5. Second, the methods disclosed in Felgner and Wheeler produce lipid/nucleic acid *complexes* in contrast to the lipid-*encapsulated* nucleic acid *particles* of our invention. This is significant for a number of reasons, many of which are already outlined in the specification of this application. In our invention the nucleic acids are *fully encapsulated* within the lipid membrane, which protects them from nuclease degradation upon *in vivo* administration and thus makes systemic administration more effective and more practical. In my view the prior art lipid/nucleic acid complexes or "aggregates" (see page 13 of the specification) of Felgner and Wheeler are unsatisfactory for this purpose since they are not serum stable, and thus they will decompose rapidly upon administration and much of the nucleic acid payload will be degraded before it can have therapeutic effect.

6. The problem of *in vivo* serum stability has been a long-standing one in the field of liposomal delivery vehicles. As discussed in our patent specification, the lipid-encapsulated nucleic acid particles of our invention solve this long-standing problem in the art. For immune stimulation in particular, adequate circulation time of the nucleic acid delivery vehicle is critical in order to achieve the desired objective of a systemic immune response. The prior art lipid/DNA complexes are inadequate for this purpose, as evidenced by some of our more recent data presented below, and as reported by a number of researchers in more recent journal articles.

7. Additional structural advantages of our lipid-encapsulated nucleic acid particles include their smaller size, generally having a mean diameter of about 50-200 nm, and the ability to achieve more uniformity in sizing, both of which are important features for intravenous formulation and administration. The lipid/nucleic acid complexes proposed by Felgner *et al.* and Wheeler *et al.* are deficient in these respects as well. For example, for the DODAC:DOPE/oligonucleotide complexes detailed below, which were prepared based on the teachings of the cited art, the size of the resulting complexes was 240 + 80 nm in diameter as measured by quasi-elastic light scattering using a Nicomp Model 370 particle sizer. Moreover, these lipid complexes are not well defined in size but consist of a "complex" of vesicle aggregates that are unstable over time.

8. I recognize Felgner *et al.* suggest generally at column 24 that "[t]he science of forming liposomes is now well developed. . . . The aqueous portion is used in the present invention to contain the polynucleotide material to be delivered to the target cell." As a skilled artisan, however, I understand the actual disclosure in Felgner to relate to lipid/DNA *complexes* which are inadequate and inferior for systemic immune stimulation for the reasons mentioned above. I do not find any discussion in Felgner *et al.* that would teach one to fully encapsulate an immunostimulatory nucleic acid as contemplated by our invention, and there is clearly no recognition by Felgner *et al.* of the surprising and synergistic effects of lipid-encapsulated oligonucleotides in immune stimulation.

9. I further recognize that Krieg *et al.* generally refer to the possibility of "associating" their immunostimulatory nucleic acids with lipids. Importantly, however, there is no teaching, suggestion or scientific basis provided by Krieg *et al.* to select lipids as opposed to the sterols or target cell specific binding agents also proposed, nor is there any basis given to fully encapsulate the nucleic acid as opposed to the standard approach of lipid complexes since both are mentioned. [Compare col. 12 with col. 33] Moreover, as with Felgner *et al.* there is certainly no suggestion in Krieg *et al.* of the surprising immune stimulatory properties that result from fully encapsulating nucleic acids in cationic lipid particles, since it was never actually accomplished in either reference.

10. We have developed additional *in vivo* data in tumor models that further demonstrates the effectiveness of our lipid encapsulation approach in comparison with lipid/nucleic acid

complexes, utilizing a complex much like those of Felgner *et al.* The cationic vesicles complexes were prepared using DOPE and the cationic lipid DODAC by dissolving the two lipids in chloroform at a 1:1 mole ratio and removing the solvent under a stream of nitrogen gas. The lipid film was then placed under vacuum to remove residual chloroform for > 4 h, protected from light before being hydrated with distilled water at a concentration of 40 mM lipid, and subjected to 5 freeze-thaw cycles using liquid nitrogen and warm (~50°C) water. The resulting multilamellar vesicles were extruded 10 times through two stacked 100 nm pore-size filters using an Extruder (Northern Lipids, Vancouver). The oligonucleotide 6295PS was mixed with the DODAC:DOPE vesicles in distilled water at a 1:1 vol/vol ratio at a concentration of 0.5 mg/ml oligonucleotide and at a cationic lipid/oligonucleotide charge (+/-) ratio of 0.15. Briefly, the 6295PS oligonucleotide, solubilized in distilled water, was pre-heated for 3 min at 80°C to convert quadruplexes and any higher ordered structures to the monomer form. The oligonucleotide (1 mg/ml, 0.375 ml) was then mixed quickly with an equal volume of the DODAC:DOPE vesicles (diluted to 12.8 mM) and allowed to incubate at room temperature for 30 minutes. The vesicle/oligonucleotide complexes were made within 4-6 hours of injection.

11. The same 6295PS oligonucleotide was also encapsulated in various cationic lipid vesicles made using DSPC, POPC or the classic composition DSPC:CH:DODAP:PEG-Cer-C14 at 20:45:25:10 as described in the instant application.

12. The figures attached to this declaration show the dramatically improved effect of lipid encapsulation in comparison with lipid/nucleic acid complexes. Figure 1 shows an effect on the stimulation of tetramer / CD8+ lymphocyte proliferation by ovalbumin. MHC-tetramer analysis is designed to detect CD8+ve, cytotoxic T lymphocytes (CTLs) that possess the appropriate T cell receptor to recognize and lyse target cells bearing the target antigen in the context of a MHC Class I complex. The lipid/nucleic acid complexes showed at best only a marginal increase in such CTLs in comparison with the control sample of ovalbumin alone. In contrast, the encapsulated oligonucleotides were at least four- to ten-fold more active in stimulating proliferation of responsive CTLs.

13. Figure 2 shows that the lipid/nucleic complexes had no effect (less than even the control) on the CTL response to target cells in a standard chromium release assay. In contrast, each of the lipid-encapsulated nucleic acids demonstrated a significant stimulation in the CTL response.

14. Figures 3 and 4 show the dramatically improved efficacy of our lipid-encapsulated nucleic acid particles in an established tumor model. As shown in Figure 3, at either 19 or 30 days after treatment, lipid/nucleic acid complexes reduced tumor volume much less efficaciously than a fully-encapsulated form of the same oligonucleotide. Similarly, Figure 4 shows that the lipid-encapsulated nucleic acid particles were much more effective in rendering the test animals tumor-free than the lipid/nucleic acid complexes.

15. The profound differences in immunostimulatory activity and tumor efficacy shown by this data are further proof of the significant immune stimulatory properties resulting from our lipid encapsulation approach. As we stated and demonstrated in our application, these immune stimulatory properties are evident even where 1) the nucleic acid is not sequence-specific (*i.e.* other than an antisense or gene expression sequence) and/or 2) the nucleic acid itself is not immunostimulatory. The significant immunostimulatory benefits of our invention went unrecognized by the prior art researchers, since they either employed inferior lipid/nucleic acid complexes (*e.g.* Felgner *et al.* and Dow *et al.*) or they merely generalized about potential lipid delivery possibilities without actually accomplishing any of them (*e.g.* Krieg *et al.*).

16. The aforementioned statements are true or based upon facts believed by me to be true. This declaration is made under penalty of perjury and with knowledge that false or misleading statements may jeopardize the invalidity or unenforceability of any rights in a patent that may be granted on the above-referenced application.

Michael J. Hope

June 23rd, 2004

Date

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